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Acute Effects of Cadmium on the Pregnant Rat and Embryo-Fetal Development

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In rats, of the Wistar-Porton strain, a single intravenous injection of 1.25 mg Cd^{2+} between days 9 and 15 of gestation results in a high incidence (80% of hydrocephalus, together with other malformations in the fetuses, examined on day 20. This dose is critical, since 1.1 mg Cd^{2+}/kg is not teratogenic, while 1.35 mg Cd^{2+}/kg kills all the embryos. Intravenous injection of Cd^{2+} to the pregnant rat on day 12 causes a dose-dependent inhibition of placental Zn^{2+} transport. At the teratogenic dose, Zn^{2+} transport is inhibited by about 75% at 4 hr. Thereafter, inhibition decreases with time but is still significant at 48 hr. At 20 hr after administration of Cd^{2+} the embryonic concentration of Zn^{2+} is depressed by 33%. In the whole embryo the activity of the Zn^{2+}-dependent thymidine kinase is inhibited by about 60% at 4 hr and at 20 hr the DNA concentration is reduced significantly. Placental transport of ^{14}C-leucine and ^{14}C-uridine, as well as the embryonic incorporation of these precursors into protein and RNA is unaffected at least at short times after the administration of Cd^{2+}. It is possible therefore, that the teratogenic effects of Cd^{2+} may be related to the inhibition of DNA synthesis in the embryo.

Pregnancy represents a period of high susceptibility to parenterally administered cadmium (Cd^{2+}). In rats, Parizek (*1*, *2*) has shown that a single subcutaneous injection of 40 μmole $CdCl_2$/kg body weight on days 17 to 21 of gestation, results in destruction of the fetal portion of the placenta with death of the embryos in a high proportion of pregnant animals. In female rats of the Wistar-Porton strain used in our laboratory which have a gestation period of 21 days, the intravenous LD_{50} drops significantly from the virgin female level of 1.77 mg to 1.05 mg Cd^{2+}/kg body weight on day 20 of gestation ($p < 0.001$; df = 30). Immediately after the administration of the LD_{50} dose, both gravid and nongravid animals develop flushed extremities, due to vasodilatation, rapid shallow respiration, apathy and flaccidity of muscles. At this dose, most deaths occur between 16 and 24 hr. With higher doses most of the animals succumb within 6 hr, the predominant pathological lesion being subpleural hemorrhage of the lung. Just before death these animals develop violent seizures. Palpation, immediately after the animal has stopped breathing reveals that the heart is still beating, and this finding suggests respiratory paralysis. Both this respiratory failure and the initial muscular flaccidity probably are due to neuromuscular block. Such effects of Cd^{2+} have been observed by Forshaw (*3*) in isolated preparation of the rat hemidiaphragm and seem to result from a competitive displacement of calcium.

Pregnant animals that die between 16 and 24 hr after the administration of the LD_{50} dose of Cd^{2+} show vaginal bleeding at 8 hr, indicative of placental damage. Histology at this stage shows degeneration of the maternal part of the placenta (Fig. 1), which is in contrast to Parizek's findings (*1*, *2*) that subcutaneous injection of Cd^{2+} salts is followed in all cases by rapidly progressive placental changes, chiefly of pars fetalis. The liver and the kidneys are edematous and hyperemic. The fetuses are pale in color and in distress, as shown by the presence of meconium-stained liquor amnii. At 20 hr the maternal animal is extremely pale, with severe hemorrhage per vaginum. Histology reveals that the placenta has lost its architecture and, as observed by Parizek (*1*), has turned into an extensive blood clot (Fig. 2). The liver exhibits periacinar necrosis, and the kidneys show vacuolation of the cells of the proximal convoluted tubules. Many areas in the

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cortex contain dilated tubules with flattened epithelium, and sometimes homogeneous casts that are probably proteinaceous. There are areas with complete degeneration of the proximal convoluted tubules. Inflammatory cells are never in large numbers but are scattered in areas containing altered tubules. The glomeruli and the distal convoluted tubules are spared. Thus, death at this stage may be due to a combination of retroplacental hemorrhage and hepatorenal failure. Nongravid females do not show these hepatic and renal changes at this dose (1.05 mg Cd^{2+}/kg), though the lungs show occasional subpleural hemorrhage.

In addition to its increased toxicity at or near term, Cd^{2+} is known to cause congenital deformities in rats, mice, and hamsters (*4–7*), when administered during a particular period of gestation. In the present work a single intravenous dose of 1.25 mg Cd^{2+} between days 8 and 15 of gestation was found to be extremely teratogenic and to induce one or more deformities in a high percentage of the fetuses (Table 1). The most frequent of these deformities is hydrocephalus, which is seen in 80% of the fetuses when examined, after delivery by cesarean section on day 20.

In addition, anophthalmia, microphthalmia, gastroschiasis, and umbilical hernia are present in 45% of the fetuses. Though the incidence of hydrocephalus is highest when Cd^{2+} is given on day 10, this deformity also occurs in a great number of fetuses when the cation is administered on day 12 of gestation. As, at this stage of development, the embryos are of reasonable size, most of the following biochemical work has been done with animals treated with a dose of 1.25 mg Cd^{2+}/kg body weight on day 12 of gestation. This dose is critical, since 1.10 mg does not produce malformations, while 1.35 mg Cd^{2+}/kg kills all the embryos.

After the intravenous injection of isotopically labelled Cd^{2+} (1.25 mg/kg) to the pregnant rat on day 12 of gestation, extremely small amounts of Cd^{2+}, i.e., about 16 ng/g wet weight tissue, are detectable in the embryos at 4 hr. At 24 hr, the amount is even smaller—about 5 ng Cd^{2+}/g wet weight tissue. At these times the corresponding total placental concentrations of Cd^{2+} are 2 and 0.8 μg/g wet weight,

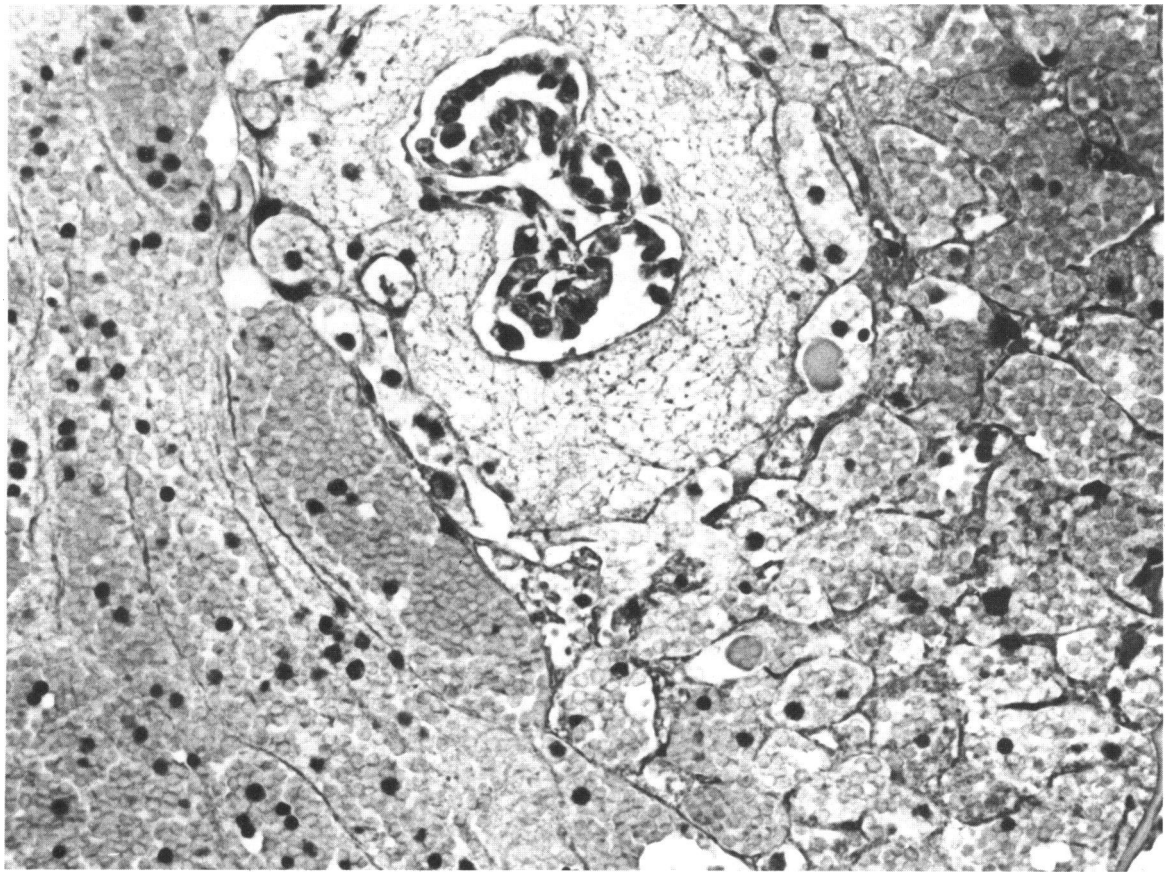

FIGURE 1. Placenta from a 20-day pregnant rat 8 hr after an intravenous injection of 1.25 mg Cd^{2+}/kg. The maternal blood vessels have undergone degeneration and the villi (see bottom of the picture) are spared.

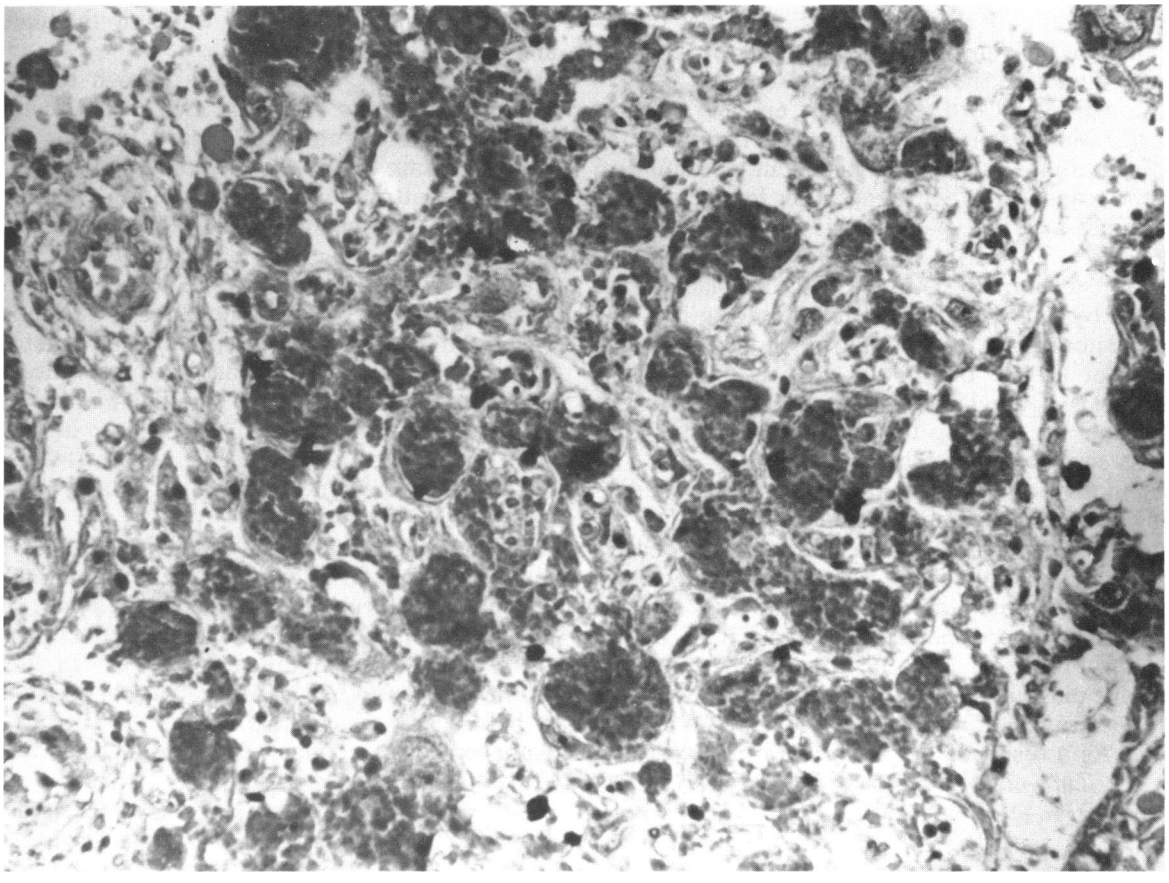

FIGURE 2. Placenta from a 20-day pregnant rat 24 hr after an intravenous injection of 1.25 mg Cd^{2+}/kg. Note the loss of placental architecture.

Table 1. Effects of intravenous injection of 1.25 mg Cd^{2+}/kg body weight on different days of gestation in the rat.[a]

Day of gestation	No. of litters	Average live per litter	% Dead or resorbed	Average fetal weight, g	No. of abnormal litters	No. of abnormal fetuses	Anomalies in all litters		
							Brain	Eye	Others
8	15	12	12.5	2.5	10	96	67	32	14
9	15	13	14.2	2.5	12	176	156	41	28
10	15	12	15.1	3.1	13	160	144	29	17
11	15	9	14.7	3.0	13	112	64	31	26
12	15	10	16.4	3.2	11	96	41	37	24
13	15	10	14.1	3.0	12	64	9	16	48
14	15	8	20.0	3.1	12	52	—	—	67
15	15	7	26.2	2.9	11	24	—	—	34

[a] The maternal animals killed and examined on the 20th day of gestation.

respectively; i.e., 100–150 times greater than the embryonic levels. This loss of Cd^{2+} from the 12-day embryo may be due at least in part to the absence of the high affinity Cd^{2+}-binding protein, thionein, since the development of the liver and kidneys is just beginning at this time. Neither zinc-thionein nor cadmium-thionein is present in the 12-day embryos from normal and Cd^{2+}-treated dams, respectively. In contrast, at 20 days the liver and kidneys of the normal fetus are well developed and the hepatic concentration of zinc-thionein is extremely high. When Cd^{2+} is administered to the mother at this stage of gestation, uptake of the cation by the fetus is greater at 24 hr than at 4 hr and, under these conditions, most of the cadmium that reaches the fetus is accumulated in the liver, specifically in the metallothionein fraction.

In agreement with these analytical measurements, $^{109}Cd^{2+}$ can be detected by autoradiography in the livers of fetuses from pregnant animals

that are dosed with the labeled cation on day 20 of gestation, but not in the whole embryos of females that are treated in the same way on day 12.

Although little Cd^{2+} reaches the embryo on day 12 of gestation, its presence in the placenta seems to interfere with the function of this organ, particularly with the transport of Zn^{2+}; a cation for which the embryo has a high demand at this stage of development (*8, 9*). Thus at 4 hr after a dose of 0.5 mg Cd^{2+}/kg, transport of $^{65}Zn^{2+}$ across the placenta is inhibited by nearly 50%, and at 1.25 mg/kg it is reduced to about 25% of the control values (Table 2). Even after a dose of Cd^{2+} as low as 0.25 mg/kg inhibition is appreciable, although not statistically significant. At 1.25 mg the inhibition persists and remains significant at 48 hr.

Quantitative interpretation of these results is difficult because of the changes caused by Cd^{2+} in the concentrations of Zn^{2+} in the plasma and other body compartments of the maternal animal. Nevertheless, as intravenous administration of Cd^{2+} results in an initial dose dependent depression of blood Zn^{2+}, the effects of Cd^{2+} on total Zn^{2+} transport across the placenta are likely to be greater, not less, than those revealed by the measurements of $^{65}Zn^{2+}$ uptake. As a result of the inhibition of Zn^{2+} transport, the Zn^{2+} concentration of the embryo is reduced by 33% at 20 hr after the intravenous injection of 1.25 mg Cd^{2+}/kg body weight to the maternal animal.

Maternal Zn^{2+} deficiency is known to lead to teratogenic effects in the rat (*8, 10*) and to reduce thymidine incorporation (*11*), particularly in the developing brain of the 12-day embryo. Such effects have been attributed to the inhibition of the Zn^{2+}-dependent enzyme, thymidine kinase. When Cd^{2+} (1.25 mg/kg body weight) is administered to 12-day pregnant rats, followed at 4, 20, and 48 hr by a 15-min pulse of ^{14}C thymidine (3 μCi), the transport of the latter at any time is found to be unaffected. Incorporation of thymidine into the embryonic DNA, however, is inhibited significantly at 4 and 20 hr although at 48 hr it is similar to that in the controls (Table 3). The total DNA content of the embryo is little affected at 4 hr but at 20 and 48 hr it is reduced significantly.

Since Cd^{2+} blocks both thymidine incorporation into embryonic DNA and also the placental transfer of Zn^{2+}, the former may be a direct result of the latter. Thymidine incorporation into embryonic DNA, however, is inhibited as early as 4 hr after the administration of Cd^{2+} to the maternal animal, that is before any measurable change has occurred in embryonic Zn^{2+} concentration. Furthermore, the effect of Cd^{2+} on placental Zn^{2+} transport is still apparent at 48 hr, whereas DNA synthesis has returned to the control level at this time. It is possible, therefore, that the small amounts of Cd^{2+} that are incorporated into the 12-day embryo have a direct inhibitory action on thymidine kinase. *In vitro,* this enzyme is known to be inhibited by Cd^{2+} (*12*). Inhibition of embryonic DNA synthesis, therefore, may be related to the teratogenic effects of Cd^{2+}, particularly as the uptake and incorporation of ^{14}C-uridine and ^{14}C-leucine by the embryos are not affected at 4 hr after the administration of Cd^{2+}. Thus, at a time when DNA synthesis is inhibited appreciably, RNA and protein production are unaffected.

Table 2. Effect of cadmium on embryonic uptake of ^{65}Zn on day 12 of gestation in the rat.

Cd^{2+} dose, mg/kg	Maternal blood-Zn^{2+} μg/g	Embryonic ^{65}Zn uptake[a]	
		μg/g	% of control
0	7.4	4.9	100.0
0.25	6.2	3.9	80.0
0.50	—	2.6	53.1
0.75	—	2.2	44.9
1.00	—	1.7	34.7
1.25	5.6	1.3	26.5

[a] Uptake measured at 15 min after the I.V. administration of $^{65}ZnCl_2$ (3 μCi) to the maternal animal.

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Table 3. Changes in thymidine incorporation and DNA concentration in the rat embryo following the administration of Cd^{2+} to the maternal animal.

Time after administration of Cd^{2+}, hr[a]	^{14}C-Thymidine incorporation into embryonic DNA, counts/min/mg DNA[b]		DNA concentration, μg/mg wet weight tissue[b]	
	Cd^{2+} treated	Control	Cd^{2+} treated	Control
4	4800	12850	4.33	4.42
20	1400	5900	3.42	4.20
48	4800	5200	3.52	4.35

[a] Rats were dosed with Cd^{2+} (1.25 mg/kg) on day 12 of pregnancy.
[b] Each value is the mean of three determinations.

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